II. REMARKS

Claim 18 has been canceled without prejudice. Claims 1, 4-6 and 17 have been amended, and new claim 19 has been added. In particular, claims 1 and 17 have been amended to recite "the plant material is transformed protonema tissue that produces heterologous proteinaceous substances" as supported on page 9, lines 32-36, and on page 15, lines 23-33, of the specification as originally filed.

Claims 4-6 have been amended to address an issue of grammar. The present amendment has no limiting effect on the scope of these claims.

New independent claim 19 corresponds to previous claim 18 rewritten in independent form. New independent claim 19 also includes the amendment wherein "the plant material is transformed protonema tissue that produces heterologous proteinaceous substances" as supported on page 9, lines 32-36, and on page 15, lines 23-33, of the specification as originally filed.

No new matter has been added to the present application by the amendment.

A. The Invention

The present invention pertains broadly to a method for producing heterologous substances in plant material, specifically cultured transformed protonema tissue, and obtaining heterologous substances produced by the transformed protonema tissue from culture medium without disrupting producing tissues or cells. In one method embodiment of the present invention, a method for the production of heterologous proteinaceous substances in plant material tissue, is provided having the steps recited in claim 1.

In accordance with another method embodiment of the present invention, a method for the production of heterologous proteinaceous substances in transformed protonema tissue from *Physcomitrella* is provided having the steps recited in claim 17.

In accordance with yet another method embodiment of the present invention, a method for the production of heterologous proteinaceous substances in transformed protonema tissue from *Physcomitrella patens* is provided having the steps recited in claim 19.

Various other embodiments in accordance with the present invention are recited in the dependent claims. All of the embodiments in accordance with the present invention provide a method for producing heterologous proteinaceous substances in plant material, whether protonema moss tissue or protonema liverwort tissue, wherein the proteinaceous substances produced by the transformed protonema tissue are advantageously obtained without disrupting producing tissues or cells.

Persons skilled in the art would recognize that the present invention advantageously utilizes protonema tissue, wherein "protonema" is defined in the art as "the usually filamentous thalloid stage of the gametophyte in mosses and in some liverworts comparable to the prothallium in ferns" (See Webster's new collegiate dictionary, 1977, p. 927)(of record).

The novelty of the present invention over the prior art relates to the step of "obtaining the heterologous proteinaceous substances...without disrupting producing tissues or cells" because persons skilled in the art would not have predicted that proteinaceous substances could be obtained from transformed protonema tissue, i.e., mature plant tissue having cell walls, without disrupting the producing tissues or cells.

B. The Rejection

Claims 1-6 and 17 stand rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement.

Claims 4-6 stand rejected under 35, U.S. C. § 112, second paragraph, as indefinite.

Claims 1-5, 17 and 18 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Houba-Hérin et al. (Nichole Houba-Hérin et al., *Cytokinin oxidase for Zea mays: purification, cDNA cloning and expression in moss protoplasts*. 17 The Plant Journal 615, 615-626 (1999))(hereafter, the "Houba-Hérin article") in view of Reutter et al. (K. Reutter and R. Reski, *Production of a heterologous protein in bioreactor cultures of fully differentiated moss plants*. 2 Plant Tissue Culture and Biotechnology 142, 142-147 (1996))(hereafter, the "Reutter article").

Claim 6 stands rejected under 35 U.S.C. § 103(a) as unpatentable over the Houba-Hérin article in view of the Reutter article, and further in view of Nasu et al. (M. Nasu et al., *Efficient transformation of Marchantia polymorpha that is haploid and has very small genome DNA*. 84 J. Ferm. Bioengin. 519, 519-523 (1997))(hereafter, the "Nasu article").

In view of the present amendment, Applicants respectfully traverse the rejection and request reconsideration for the following reasons.

C. Applicants' Arguments

In view of the present amendment, claims 1-6, 17 and 19 are in compliance with 35 U.S.C. § 112, second paragraph.

Claims 1-6, 17 and 19 also comply with 35 U.S.C. § 112, first paragraph. Applicants' assert that the Examiner has not established a prima facie case of lack of enablement under 35 U.S.C. § 112, first paragraph, because the present invention pertains to a method of culturing of transgenic bryophyte protonema (i.e., from mosses and liverworts) that produce heterologous proteinaceous substances and of obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. The Examiner erroneously emphasizes the scope of genetic transformation of the protonema tissue employed in the presently claimed invention as the basis for the Section 112 rejection.

The Examiner has failed to recognize that the present invention pertains to a method comprising the steps of (a) culturing...transformed protonema tissue that produces heterologous proteinaceous substances and (b) obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. Instead, the Examiner appears to be interpreting the claims to include steps (a) and (b), and the step of "transforming" protoplast cells (Office Action dated June 7, 2005, at 4, lines 16-20). However, the transformation of bryophyte protoplasts is well known in the art for many species of bryophytes, and is not an element of the claim. Rather, the present invention focuses on the culturing step and the step of obtaining heterologous proteinaceous substances produced by transformed protonema tissues.

i. The Examiner's Rejection Under 35 U.S.C. § 112, First Paragraph

The Examiner admits that the present application is enabling for a method for the production of proteins in *Physcomitrella patens* (Office Action dated October 4, 2004, at 2, lines 19-20; and Office Action dated June 7, 2005, at 2, lines 15-17), which is a bryophyte. For this reason, the Examiner's enablement rejection cannot apply to new claim 19.

However, the Examiner asserts that no other bryophytes, not even any of the other five *Physcomitrella* species, are reasonably enabled by the present disclosure on the grounds that application of the heterologous gene in accordance with the present invention to any other bryophyte would require undue trial and error experimentation (Office Action dated October 4, 2004, at 3, lines 10-13; and Office Action dated June 7, 2005, at 2, line 18, to at 3, line 2). On the other hand, the Examiner admits that (i) "Zeidler et al. disclose transformation of the moss *Ceratodon purpureus* (pg 643-647)" (October 4th Office Action, p. 6, lines 17-19) and also that (ii) "Nasu et al. teach transformation of *Marchantia polymorpha* (pg 520, left column, paragraphs 1-2)," which is a liverwort (October 4th Office Action, at 6, lines 1-6).

Examiner's arguments are contradictory. Specifically, the Examiner admits that the present invention is enabled for *Physcomitrella patens* and then points out based on the prior art that a person of ordinary skill in the art would know how to genetically transform additional moss species (i.e., *Ceratodon purpureus*) and additional liverwort species (i.e., *Marchantia polymorpha*). The Examiner also argues that it would be obvious to do so on the grounds that "substitution of one bryophyte for another is an obvious optimization of design parameter" (October 4th Office Action, at 6, lines 10-12; and Office Action dated June 7, 2005, at 7, lines 18-19). The Examiner has in fact correctly established, based on her citation of the prior art, that the transformation of mosses and liverworts is well known and predictable.

Applicants do not rely on argument alone, but on facts. Attached herewith is a Declaration under 37 C.F.R. § 1.132 by Gilbert Gorr (hereafter, the "Gorr Declaration"), co-inventor of the above-captioned application and an internationally recognized researcher in the field of bryophyte biology. According to the testimony of Dr. Gorr, there is no reason a person skilled in the art would not expect other members of the *Physcomitrella* species to behave in the same way under the same cell culture conditions as *Physcomitrella patens* (Gorr Declaration, ¶¶ 5 and 6). Furthermore, the experimental evidence provided by the Gorr Declaration shows that more distant bryophyte relatives, such as *Funaria hygrometrica* (a moss) and *Marchantia polymorpha* (a liverwort), can be successfully transformed by routine experimentation using the transformation protocol described in the instant application (Gorr Declaration, ¶¶ 15-18). From these facts, Dr. Gorr's testimony establishes that cultivation and transformation of bryophytes, including both mosses and liverworts, is mature and well developed, and that a person of ordinary skill in the art would have known, at the time the invention was made, how to transform and culture other *Physcomitrella* species, *Ceratodon* species, *Marchantia* species, and many

other species of bryophytes as well because "bryophytes are simple, primitive plants that are expected to behave biologically in a relatively uniform manner" (Gorr Declaration, ¶ 30).

In view of the facts admitted by the Examiner, and the additional facts established by the Gorr Declaration, Applicants traverse the Examiner's grounds for the present lack of enablement rejection under 35 U.S.C. § 112 standing against the instant claims because the Examiner has produced no evidence grounded in the prior art in support of this rejection, and has evinced no sustainable reason for the rejection. It is the Examiner's burden to set forth a reasonable explanation as to why the scope of protection sought is not enabled by the description of the invention. In re Wright, 27 U.S.P.Q.2d 1510, 1513 (Fed. Cir. 1993). Specifically, the Examiner has provided no evidence (i.e., article, textbook, technical disclosure, etc.) to show, or even suggest, that by enabling the invention for *Physcomitrella patens* (as has been admitted by the Examiner) the Applicants have not enabled the invention for any other species or subspecies of bryophyte.

On the contrary, the evidence adduced by the Examiner based on the prior art of record, such as Nasu article, actually supports the conclusion that the present invention is enabled for both mosses and liverworts. This is not a case where the Examiner has provided some evidence, such as a published article, to establish the unpredictability of the target class of organisms. See In re Vaeck, 20 U.S.P.Q2d 1438, 1445 (Fed. Cir. 1991)(Claimed subject matter related to transforming cyanobacteria, a diverse and relatively poorly understood group of microorganisms); and In re Wright, 27 U.S.P.Q.2d 1510, 1513-4 (Fed. Cir. 1993)(Examiner produced an article teaching that the physiologic activity of RNA viruses was sufficiently unpredictable to establish that all living organisms could be immunized against infection by any pathogenic RNA virus by inoculation with a live virus containing the antigenic code, but not the pathogenic code, where applicant provided only one working example).

This is a case where the Examiner has provided absolutely no evidence to show, or even suggest, that bryophytes are poorly understood and/or unpredictable organisms. In the present case, the Examiner has simply failed to establish a prima facie case of lack of enablement under 35 U.S.C. § 112, as a matter of law, because the Examiner has provided no reasonable basis for the rejection.

ii. The Elements of Enablement

The statutory enablement requirement of 35 U.S.C. § 112, first paragraph, is a question of law pertaining to whether a specification teaches those of ordinary skill in the art how to make and use the full scope of the claimed invention without undue experimentation. In re Wright, 27 U.S.P.Q.2d at 1513. The initial burden rests on the Examiner to provide sufficient reasons for doubting assertions in the specification as to the scope of enablement. Id.

In the present case, the Examiner admits the specification is enabling to those of ordinary skill in the art for the bryophyte *Physcomitrella patens*, and the Examiner has produced evidence that a person skilled in the art would know how to transform *Ceratodon purpureus* and *Marchantia polymorpha* without undue experimentation. The wealth of information uncovered by the Examiner teaches that the field of bryophyte transformation is mature, well developed and predictable. This same conclusion follows from Dr. Gorr's review of relevant scientific literature and from the results of additional experiments demonstrating the ease of transforming other bryophyte species (Gorr Declaration, ¶ 30).

Despite overwhelming evidence to the contrary, the Examiner asserts that the heterologous gene inserted into a single species of bryophyte would not enable an ordinary person skilled in the art to practice the claimed invention without undue experimentation in any other bryophytes, not even closely related mosses such as *Funaria*, *Sphagnum*, *Ceratodon* and

other *Physcomitrella* species (October 4th Office Action, at 3, lines 3-13; Office Action of June 7, 2005, at 2, line 15, to at 3, line 12). The Examiner's argument necessarily fails in view of the Examiner's admission that it is well known in the art to transform mosses, such as *Ceratodon*, and liverworts, such as *Marchantia*, and the additional experimental evidence submitted in the Gorr Declaration demonstrating how easy it is to transform other mosses, such as *Funaria hygrometrica*, and liverworts, such as *Marchantia polymorpha* (Gorr Declaration, ¶¶ 14-18).

The Federal Courts have ruled that every species in a genus encompassed by the claims need not be disclosed in order to meet the enablement requirement for the genus; however, the enablement analysis must be made on a case-by-case basis. In re Angstadt, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976). Enablement is not precluded by the necessity for some experimentation; rather, the question is whether the amount of experimentation is "undue experimentation" as determined upon the weighing of many factors. In re Wands, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988). Factors to consider when determining whether a disclosure would require undue experimentation include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. Id.

The Examiner contends that the specification does not enable the claimed genus (in the patent law sense) because the amount of experimentation required to transform other mosses and liverworts would amount to "undue trial and error experimentation." (Office Action, dated October 4, 2004, page 3, lines 10-13). Applicants disagree for the following reasons.

iii. Analysis of the Wands Factors for Claims Reciting the Protonema Tissue

Claim 1 recites a "method for the production of heterologous proteinaceous substances in plant material...wherein the plant material is transformed protonema tissue....". An analysis of the Wands Factors for methods for the production of heterologous proteinaceous substances in plant material in accordance with claims 1-6 is as follows.

Direction and Guidance from Specification

First, the present specification gives ample guidance regarding how to practice and use the claimed invention as recited in claims 1-6. Specifically, the specification provides ample guidance regarding how to use intact plants to obtain heterologous proteinaceous substances directly from the culture medium without disrupting producing tissues or cells (See specification, page 9, line 5, to page 27, line 26). In other words, the present specification provides about 18 pages of detailed instruction.

Also, well-known and commercially available starting materials for practicing the methods are known in the prior art (see page 7, lines 1-8, and page 9, lines 6-24), with materials for a detailed example described on page 11, line 29, to page 26, line 18, of the present specification. In particular, the specification describes multiple species of mosses and liverworts that are well-known, characterized, and previously studied and that are suitable for practicing the present invention, including *Physcomitrella*, *Funaria*, and *Ceratodon* species (See specification, page 9, lines 6-24, and R. Reski, *Development, genetics and molecular biology of mosses.* 111 Bot. Acta 1, 1-15 (1998)(of record), hereafter the "Reski article") as well as *Sphagnum* species (See specification, page 9, lines 6-18, and H. Rudolph and S. Rasmussen, *Studies on secondary metabolism of Sphagnum cultivated bioreactors.* 3 Crypt. Bot. 67, 67-73 (1992)(of record), hereafter the "Rasmussen article").

Furthermore, the specification gives detailed direction and guidance regarding the genetic transformation of the species *Physcomitrella patens* so that its protonema tissue produces vascular endothelial growth factor VEGF (See specification as originally filed, page 11, line 29, to page 18, line 35). The specification explains that genetic transformation systems for *Physcomitrella patens* have been previously developed and described for enzyme production (See specification, page 9, line 26, to page 10, line 2, and K. Reutter and R. Reski, *Production of heterologous protein in bioreactor cultures of fully differentiated moss plants.* 2 Plant Tissue Culture and Biotechnology 142, 142-147 (1996)(of record)) and for producing cytokines (See N. Houba-Herin et al., *Cytokinin oxidase from Zea mays: purification, cDNA cloning and expression in moss protoplasts.* 17 The Plant Journal 615, 615-626 (1999)(of record)).

In view of the above facts demonstrating the application of gene transformation techniques to multiple species of plant protonema tissue, it is evident that a person of ordinary skill in the art would only have to apply routine experimentation to adapt the method described by the specific example provided by the instant specification, wherein the plant protonema tissue is *Physcomitrella patens*, to other species of protonema tissue whether mosses or liverworts. This conclusion, based on the direction and guidance provided by the disclosure of the above-captioned application, is bolstered by the fact that when such additional experiments are carried out by those of ordinary skill in the art, successful transformation of other species of mosses and liverworts can be achieved without undue experimentation (See Gorr Declaration, ¶¶ 18 and 30).

Thus, the first Wands factor weighs in favor of enablement.

Working Example Present

Second, the present specification provides a specific enabling example in the disclosure to teach how to perform and use the "method for the production of heterologous proteinaceous substances in plant material...wherein the plant material is protonema tissue" as described on page 11, line 29, to page 18, line 35 of the specification as originally filed. Protonema tissue is a well-known plant tissue type, which manifests numerous common biological characteristics and functions whether originating from moss or liverwort species. See Gorr Declaration, ¶¶ 5-22.

Thus, the second Wands factor weighs in favor of enablement.

Nature of the Invention and State of the Art

Third, the present invention is directed to a method of producing heterologous proteinaceous substances using genetically transformed protonema tissue. Thus, the method in accordance with this embodiment of the present invention employs simple plant organisms that have a well characterized plant physiology and predictable developmental cycle (See R. Reski, *Development, genetics and molecular biology of mosses.* 111 Bot. Acta 1, 3, 6 and 11 (1998); and the Nasu article, at 519, first column, lines 1-24). In other words, the biological plant organisms producing protonema tissue, whether of the moss phylum (e.g., *Physcomitrella*, *Funaria*, *Sphagnum* and *Ceratodon*) or the liverwort phylum (e.g., *Marchantia* and *Sphaerocarpos*), are relatively simple, predicatable organisms. The State of the Art is mature as evident from such prior art references as the Houba-Hérin article, the Reutter article, the Zeidler article, the Nasu article, the Rasmussen article, and the review article by H. Mühlbach, *Use of plant cell cultures in biotechnology.* 4 Biotechnology Annual Review 113, 158-161 (1998) (specifically, page 158, line 32, to page 161, line 6 (of record))(hereafter, the Mühlbach article). In fact, the Mühlbach article states that

> [t]hese studies document the advanced stage that is currently achieved in the genetic transformation of *P. patens*, which can be certainly extended to other genes and also to other bryophytes with potential use in biotechnology.
>
> Evidence along this line comes from studies on the expression of the human vascular endothelial growth factor (VEGF) protein in bioreactor cultures of *P. patens*....In general, these promising approaches clearly demonstrate the feasibility of bioreactor cultures of transgenic mosses for the production of heterologous compounds.

H. Mühlbach, at page 160, line 38, to page 161, line 6, (emphasis added).

In addition, the primary references recited against the claims of the present application, such as Houba-Hérin article, the Reutter article, the Zeidler article and the Nasu article, were published about 5 or more years ago, which further suggests the mature nature of the relevant art. Lastly, following a review of relevant scientific literature, Dr. Gorr testifies that the state of the art regarding the cultivation and transformation of bryophytes, including both mosses and liverworts, is mature and well developed (Gorr Declaration, ¶ 30).

Thus, the third Wands factor weighs in favor of enablement.

The Relative Skill of Those in the Art

Fourth, as is generally known, persons of ordinary skill in the art of transforming plant cells to express selected proteins, such as human VEGF, are highly trained professionals with advanced degrees in cellular and molecular biology who are involved in research and technological advancement of the field. The relative skill level of those in the art is notably high (See, e.g., the Curriculum Vitae of Dr. Gilbert Gorr, attached herewith).

Thus, the fourth Wands factor weighs in favor of enablement.

Predictability or Unpredictability of the Art

Fifth, Applicants can point the Examiner back to the Zeidler article and the Nasu article to show that the transformation of other protonema species, such as *Ceratodon* and *Marchantia*, are known. The predictability of the art, pertaining to the transformation of plant species that ultimately produce protonema tissue, is highly predictable. This fact is additionally supported by the Gorr Declaration, ¶¶ 11-18).

Thus, the fifth Wands factor weighs in favor of enablement.

Quantity of Experimentation Necessary

Sixth, the present specification generally outlines the method for the production of heterologous proteinaceous substances in protonema tissue in accordance with claims 1-6 of the present invention, as described on page 8, line 1, to page 18, line 35, using known and readily available materials. The crux of the present invention is obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. The method in accordance with these directions may require some experimentation in order to optimize results using protonema from species other than *Physcomitrella patens*, *Ceratodon purpureus* and *Marchantia polymorpha*; however, Applicants assert that while some experimentation may be necessary, it is no more than is commonly encountered in the art. Applicants assertion is supported by the experimental evidence submitted in the Gorr Declaration, which shows successful transformation of *Funaria hygrometrica* was achieved using the protocol described on page 14, line 1, to page 16, line 7, and on page 16, lines 28-35, of U.S. Patent Application No. 10.089,450, and that such successful transformation required no more than routine experimentation as is commonly encountered in the art (Gorr Declaration, ¶¶ 15, 16 and 21-23).

As is clearly established by the scientific references of record and by the testimony and experimental evidence provided by the Gorr Declaration, a person of ordinary skill in the art would know how to transform *Physcomitrella patens*, *Ceratodon purpureus*, *Funaria hygrometrica* and *Marchantia polymorpha* protoplasts, in view of Applicants' disclosure and the state of the art, without undue experimentation. However, transformation of plant protoplasts is not the crux of the invention. Be that as it may, transformation of other plant protoplasts of species that produce protonema tissue is a matter of routine experimentation in the art in view of the biological predictability of protonema forming plant tissues in general.

Thus, the sixth Wands factor weighs in favor of enablement.

Breadth of Claims

Seventh, the breadth of claim 1 includes the method of production of heterologous proteinaceous substances in protonema tissue. The term "protonema" has a specific meaning in the art and limits the scope of the present invention to tissues that are "the primary usually filamentous thalloid stage of the gametophyte in mosses and in some liverworts." Furthermore, the claimed method includes two steps: (i) culturing transformed protonema tissue in a culture medium to produce heterologous proteinaceous substances, and (ii) obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. The breadth of claim 1 includes the species embodiment disclosed on page 11, line 29, to page 27, line 26, and is limited to transformed bryophyte strains. The breadth of claim 1 is not overly broad.

Thus, the seventh Wands factor weighs in favor of enablement.

Summary of the Wands Factors Applied to the Protonema Tissue Embodiment

The above factors favor, as a matter of law, that Applicants' application as originally filed would teach a person of ordinary skill in the art how to make and use the claimed invention without undue experimentation. Specifically, Applicants' application provides (i) considerable direction and guidance on how to practice the invention, (ii) at least one very detailed working example of protonema tissue producing heterologous proteinaceous substances, (iii) there is an extremely high level of skill in the art, and (iv) the methods and materials needed to practice the invention are well known. While the degree of predictability in practicing the invention is not 100% (i.e., because not every possible protonema species has been studied in the prior art), multiple species of mosses and liverworts have been transformed in the prior art and by the disclosure of the present invention. Furthermore, the experimental evidence of the Gorr Declaration shows how easy it is to transform additional bryophyte species besides *Physcomitrella patens*, using no more than routine experimentation, thereby demonstrating a high degree of predictability in the art. With respect to the transformation of bryophytes, the predictability is reasonably more predictable than other biotechnological arts that have been deemed enabled under similar circumstances. In re Wands, 8 U.S.P.Q.2d at 1406. Lastly, the breadth of the independent claim 1 of the present invention covers, in scope, the genus (in the patent law sense) of protonema tissue taught in the specification. The breadth of claim 1 is not overbroad.

In short, all of the Wands factors favor enablement.

iv. The Examiner's Rejection Under 35 U.S.C. § 103(a)

A prima facie case of obviousness requires a showing that the scope and content of the prior art teaches each and every element of the claimed invention, and that the prior art provides some teaching, suggestion or motivation to combine the references to produce the claimed invention. In re Oetiker, 24 U.S.P.Q.2d 1443 (Fed. Cir. 1992); In re Vaeck, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991). In the present case, the Examiner has failed to establish a prima facie case of obviousness because the scope and content of the subject matter taught by the references is insufficient to teach, or suggest, all of the claimed elements and the references fail to provide a teaching, suggestion, or motivation for justifying the asserted combination.

The Houba-Hérin Article

The Houba-Hérin article teaches transforming moss protoplasts, in particular *Physcomitrella patens* protoplasts, to analyze cytokinin oxidase (CKO) or β-glucuronidase (GUS) activity (Houba-Hérin article, See Abstract, and page 619, col. 2, lines 21-48, and page 624, col. 2, lines 35-53).

Thus, the Houba-Hérin article teaches transiently transforming protoplasts (it doesn't teach transformed protonema tissue) and keeping them alive for a period of time, which a person skilled in the art would recognize are undifferentiated cells without cell walls (See Webster's new collegiate dictionary 927 (1977), "**protoplast**...**2 a :** the nucleus, cytoplasm, and plasma membrane of a cell constituting a living unit distinct from inert walls and inclusions"). On the other hand, the present invention obtains heterologous proteinaceous substances from "protonema tissue" as recited in claims 1 and 17, which a person skilled in the art would recognize are differentiated plant tissues (See Webster's new collegiate dictionary 927 (1977)

"**protonema**...: the primary...thalloid stage of the gametophyte in mosses"). A person of ordinary skill in the art would know that such protonema tissue has cell walls.

While the present inventors may make use of plant protoplasts when transforming cells, the present invention uses transformed protonema tissue for producing the heterologous proteinaceous substances, and it is the heterologous proteinaceous substances produced by the protonema tissue that are obtained without disrupting producing protonema tissue or cells. Thus, while plant protoplasts may be used in preparing transformed protonema tissue for practicing the presently claimed invention, it is protonema tissue that is used in accordance with the present invention for producing the heterologous proteinaceous substances. Thus, plant protoplasts are not elements of the present invention as claimed.

While the present invention includes the step of culturing transformed protonema tissue, and the present invention uses the transformed protonema tissue for producing the heterologous proteinaceous substances, the steps of transforming protoplasts and growing them into "transformed protonema tissue" are not elements of the claims. Thus, while plant protoplasts may be used in preparing transformed protonema tissue for practicing the presently claimed invention, it is culturing transformed protonema tissue that is presently claimed as a step in Applicants' "method for the production of heterologous proteinaceous substances in plant material for producing the heterologous proteinaceous substances". The process of transforming plant protoplasts and of using transformed plant protoplasts to generate transformed protonema tissue is not an element of the present invention as claimed.

Applicants further point out that a person skilled in the art could not have predicted that using protonema tissue would be a good approach for easily obtaining heterologous proteinaceous substances from the medium for the reason that the producing protonema tissue comprises cells having cell walls. Typically, proteinaceous substances made by fully

differentiated plants are trapped in a space located between the plasma membrane and the cell wall. Consequently, conventional retrieval of such substances from mature plants requires disrupting their cell wall, e.g. by mechanical or chemical means. In accordance with the invention as recited in claims 1, 17 and 19, however, the "transformed protonema tissue...produces heterologous proteinaceous substances" that can be obtained from the culture medium "without disrupting producing tissues or cells".

It is thus evident that the Houba-Hérin article does not disclose or suggest the steps, in accordance with claims 1, 17 and 19 of the present invention, of (a) culturing...transformed protonema tissue that produces heterologous proteinaceous substances, and (b) "obtaining the heterologous proteinaceous substances from the culture media without disrupting producing protonema tissues or cells."

The Reutter Article

The Reutter article teaches the transformation of moss protoplasts, in particular *Physcomitrella patens* protoplasts, using the PEG-mediated direct DNA transfer with a 11.5 kb plasmid carrying the E. *coli gus*-gene under the control of the cauliflower mosaic virus (CaMV) 35S-promotoer as well as the *npt*II-gene under control of the *Agrobacterium tumefaciens* nopalin synthase promotor (Reutter article, page 143, line 4, to page 144, line 5). The Reutter article also discloses culturing protonemata to form protonema balls using high speed stirring (Reutter article, at 145, lines 1-5). However, the Reutter article is completely silent with respect to obtaining the β-glucuronidase produced by the moss protonema balls without disrupting producing tissues or cells as recited in claims 1, 17 and 19. It can be assumed from the teachings of the article that any detected material was from lysed or disrupted cells. The purpose of

Reutter's study was not to obtain heterologous proteinaceous substances from culture media and there is no indication in their study that this was possible.

The Reutter article explicitly used an assay taught by Jefferson to quantify intracellular β-glucuronidase activity of the transformed moss plants, which necessarily involved the lysis of the transformed cells (See Reutter article, at 143, lines 23-32; and Richard A. Jefferson, *Assaying chimeric genes in plants: the GUS gene fusion system*, 5 Plant Molecular Biology Reporter 387, 392 (1987)(of record). Thus, there is no teaching in Reutter that isolation of heterologous proteinaceous substances from medium was possible, and the assay they used specifically indicates that they did not believe it was possible.

It is thus evident that the Reutter article does not disclose or suggest the steps, in accordance with claims 1, 17 and 19 of the present invention, of (a) culturing...transformed protonema tissue that produces heterologous proteinaceous substances, and (b) "obtaining the heterologous proteinaceous substances from the culture media without disrupting producing protonema tissues or cells."

The Nasu Article

The Nasu article pertains to the transformation of *Marchantia polymorpha* that is a haploid liverwort with very small genome DNA (See Abstract). In particular, the Nasu article teaches transforming single *Marchantia* cells with a binary vector plasmid pB1121 and a plasmid pRiA4b so as to inactivate the GUS gene (Nasu article, at 520, first column, lines 1-11). Thus, the Nasu article pertains to cultures of single cells, and not to intact plant tissue. The transformed cells were subsequently fixed in formaldehyde, which a person of ordinary skill in the art would realize kills the cells, and then the dead cells were stained for GUS activity (Nasu article, at 520, first column, lines 35-47, and see Figure 4).

The above characterization of the scope and content of the teachings of the Nasu article is supported by the testimony of Dr.Gorr, who states that the Nasu article does not teach that heterologous protein produced by transformed *Marchantia* cells would be secreted through the cell walls of mature protonema cells (See Gorr Declaration, ¶ 28). Thus, the Nasu article plainly does not teach or suggest the steps, in accordance with claims 1, 17 and 19 of the present invention, of (a) culturing...transformed protonema tissue that produces heterologous proteinaceous substances, and (b) "obtaining the heterologous proteinaceous substances from the culture media without disrupting producing protonema tissues or cells."

v. Summary of the Prior Art

In summary, the Houba-Hérin article teaches transiently transforming and maintaining *Physcomitrella patens* protoplasts for a short period of time in order to analyze cytokinin oxidase (CKO) or β-glucuronidase (GUS) activity, but it does not teach culturing transformed protonema tissue, and it does not teach obtaining the heterologous proteinaceous substances from this protonema tissue without disrupting producing tissues or cells.

The Reutter article teaches the stable transformation of *Physcomitrella patens* protoplasts using the PEG-mediated direct DNA transfer in order to intracellularly accumulate β-glucuronidase. While the Reutter article teaches culturing moss protonemata and protonema balls, the article is completely silent with respect to obtaining the β-glucuronidase produced by the protonema without disrupting producing tissues or cells. And in fact, the only assay used for the testing for β-glucuronidase was one that required the lysing of cells, which indicates that they did not believe that isolation of the β-glucuronidase from the culture medium was possible.

The Nasu article teaches transforming *Marchantia* cells and analyzing recombinant intracellular GUS activity by fixing plant material in formaldehyde and staining for GUS

activity. The Nasu article does not teach production of extracellular heterologous proteinaceous substances, and it does not teach obtaining the heterologous proteinaceous substances without disrupting producing tissues or cells.

Plainly, none of these prior art references reasonably teach, or even suggest, the steps of (a) "culturing ...transformed protonema tissue that produces heterologous proteinaceous substances" and (b) "obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells" as recited in independent claims 1, 17 and 19 of the present application. The scope and content of the references is insufficient to establish a prima facie case of obviousness.

To be absolutely clear regarding this point, all combinations of the Houba-Hérin article, the Reutter article, and the Nasu article would still fail to teach, or suggest, the steps of (a) "culturing ...transformed protonema tissue that produces heterologous proteinaceous substances", and (b) "obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells" as recited in independent claims 1, 17 and 19 of the present application. For the above reason alone, the Examiner's Section 103 rejection is untenable and must be withdrawn.

v. Lack of Proper Motivation to Combine and Reasonable Expectation of Success

A proper rejection under Section 103 further requires showing (1) that the prior art would have suggested to a person of ordinary skill in the art that they should make the claimed device or carry out the claimed process, (2) that the prior art would have revealed to a person of ordinary skill in the art that in so making or doing, there would have been a reasonable expectation of success, and (3) both the suggestion and the reasonable expectation of success must be found in the

prior art and not in the applicants' disclosure. In re Vaeck, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). In the present case, the Examiner has not shown that the references of record provide a suggestion to combine the references, or that combination of the references would lead to a reasonable expectation of success.

As discussed above, the Houba-Hérin article teaches a method of transiently transforming and maintaining moss protoplasts for a short period of time, whereas the Reutter article teaches a method of transforming moss protoplasts that are regenerated to the protonema stage in order to continuously produce an intracellular heterologous protein. The references do not teach how such different protocols could be combined, and the references provide no motivation for the combination as supported by the testimony of Dr. Gorr (See Gorr Declaration, ¶¶ 26 and 29).

Even assuming, *arguendo*, that the proposed combination of the teachings of the Houba-Hérin article and the Reutter article could be made (which Applicants assert is an erroneous assumption), this combination would clearly fail to teach, or suggest, a reasonable expectation that the heterologous protein could be obtained without disrupting producing tissues or cells. The teachings of the Nasu article would fail to make up any of these deficiencies.

For all of these reasons, the Examiner has failed to demonstrate a suggestion, grounded in the art of record, for making the proposed combination of teachings and the Examiner has failed to demonstrate that the references would teach a reasonable expectation of success in arriving at the presently claimed invention even if the references could be combined.

III. CONCLUSION

Claims 1-6, 17 and 19 are now in compliance with 35 U.S.C. § 112, second paragraph. Claim 19 is enabled for the reasons of record conceded by the Examiner.

Claims 1-6 and 17 pertain to a method for the production of heterologous proteinaceous substances in protonema tissue, and comply with the enablement requirement of 35 U.S.C. § 112, first paragraph, for the reasons described above. The Section 112, first paragraph, enablement rejection, however, is untenable for multiple reasons. First, the Examiner has failed to establish a prima facie case regarding lack of enablement because the Examiner has not adduced any evidence that bryophytes are biologically unpredictable, and/or poorly understood, organisms such as could even raise an issue regarding enablement. Second, the Examiner has failed to properly analyze the Wands factors, all seven of which weigh in favor of enablement. And third, the process of transforming bryophytes is not presently recited as a step of the claimed invention. Instead, the presently claimed method, in accordance with the present invention, cultures "transformed protonema tissue," but the process by which "transformed protonema tissue" is created is not a limitation of the present claims.

The rejection of claims 1-6, 17 and 18 under 35 U.S.C. § 103(a) is untenable and must be withdrawn because the scope and content of the teachings of the asserted references is insufficient to sustain the rejection. Specifically, neither the Houba-Hérin article, the Reutter article, nor the Nasu article teach, or suggest, the steps of (a) "culturing ...transformed protonema tissue that produces heterologous proteinaceous substances" and (b) "obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells" as recited in independent claims 1, 17 and 19 of the present application.

The Section 103 rejection is also untenable and should be withdrawn because the Examiner has failed to demonstrate a motivation to combine the references that is grounded in

the prior art, and the Examiner has failed to show that a person of ordinary skill in the art would have a reasonable expectation of success of arriving at the claimed invention when combining the teachings of the references.

For all of the above reasons, claims 1-6, 17 and 19 are in condition for allowance, and a prompt notice of allowance is earnestly solicited.

Questions are welcomed by the below-signed attorney for applicants.

Respectfully submitted,

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